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Erythrocyte Diapedesis in Anterior Pituitary Hemorrhage after Intraperitoneal Injection of Hypertonic Solution in Mice

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ABSTRACT—Initial changes of hemorrhage occurring in the anterior pituitary of mice received ip injection of 9% NaCl at a dose of 0.03 ml/g body weight were investigated with the electron microscope. The sinusoidal capillaries were distended and filled with erythrocytes after 10 min of the injection. Extravasation of red blood cells took place without distinct injury to the endothelium, being involved in a mechanism known as diapedesis. Two types of passages traversing the endothelium were distinguished in this erythrocyte diapedisis: through the large endothelial gaps up to 1 μm in diameter, and the other of, predominantly, through the occluding junctions of adjacent cells of the endothelium. In the latter case, the red blood cell showed thread-like configuration in the middle of the cell body when migrating across the endothelial tight junction. In addition the erythrocyte migration through the zonula occludens of the marginal cells with no obvious structural injury was encountered. The intercellular junctions of the endothelium as well as those of the marginal cells of the anterior pituitary may be provided with the zonula occludens which is very leaky in structure.

INTRODUCTION

Our previous study has demonstrated that hypertonic solution induced acute and intense hemorrhage into the anterior pituitary in mice [1]. This incidental heavy bleeding occurred exclusively in the anterior pituitary 10 min after ip injection of hypertonic solutions of electrolytes or non-electrolytes. No hemorrhage was observed in other endocrine glands and visceral organs so far investigated histologically [1].

Ultrastructural studies on experimental hemorrhage in endocrine glands and some organs have been reported. Leakage of red blood cells through the capillary wall of the anterior pituitary after electrolytic destruction of pituitary stalk [2], hemorrhagic necrosis (apoplexy) by acrylonitrile in the adrenals [3], extravasation of erythrocytes in the adrenal cortex after chronic ACTH stimulation [4] and fragmented erythrocytes in the mesangium of the rabbit kidney after the Shwartzman reaction [5]. In these studies, however, no ultrastructural findings for the initial changes of erythrocyte extravasation have been illustrated. Therefore, the present electron microscopic study was designed to elucidate the changes at the beginning of the migration of red blood cells across the endothelium in this acute pituitary hemorrhage in mice.

The results disclosed a fact that this anterior pituitary hemorrhage is involved in "diapedisis", a mechanism by which extravasation of red blood cells takes place without structural destruction of the endothelial cells.

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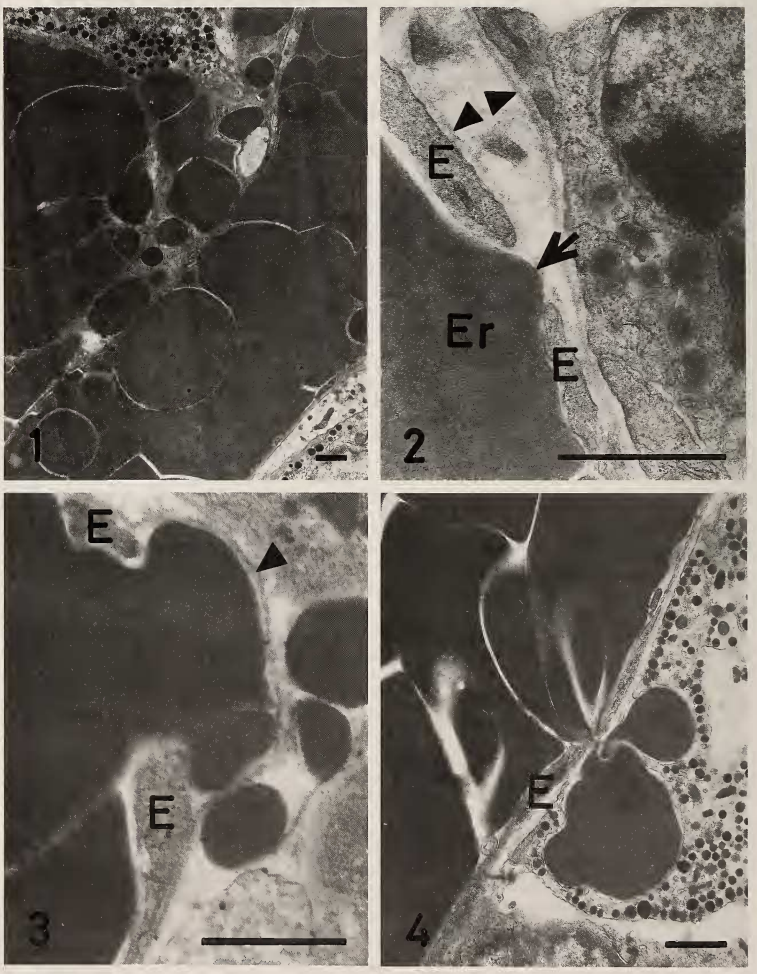
MATERIALS AND METHODS

Male mice of the Jcl/ICR strain were housed in air-conditioned room ($21 \pm 1^{\circ}$C) with lights on from 7:00 to 21:00 hr. They were fed on standard laboratory chow and were allowed free access to tap water. Ten animals at 8 weeks of age received a single ip injection of 9% NaCl at a dose of 0.03 ml/g body weight. They were sacrificed by decapitation 10 or 20 min after the injection. The pituitary glands were removed and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) followed by buffered 1% OsO_4 for 1 hr each.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Specimens were dehydrated and embedded in Quetol 812. Parasagittal ultrathin sections were stained with uranyl acetate and lead citrate, and examined with an 11-E Hitachi Electron Microscope.

RESULTS

The anterior pituitary showed acute hemorrhage 10 min after the ip injection of 9% NaCl at a dose of 0.03 ml/g body weight. However, the administration of hypertonic solution via the tail vein under ether anesthesia at the same dose failed to cause pituitary hemorrhage. Sinusoidal blood capillaries appeared to be dilated and filled with red blood cells showing apparent congestion in the anterior pituitary after the ip injection of 9% NaCl (Fig. 1). Despite such expansion of blood vessels any lesions or discontinuities of the vasculature were not observed. Neverthless, there was massive extravasation of red blood cells from capillaries into the intercellular space of the parenchymal cells of the anterior lobe (Fig. 1).

In the extravasaion of erythrocytes there seemed to be two passages across the endothelial cells in this anterior pituitary hemorrhage. In the first type of migration, erythrocytes moved through endothelial gaps and/or endotherial stomata. The small extrusion of erythrocytes was inserted into the endothelial gaps which were invested with the basal lamina (Fig. 2). Succesively, large endothelial gaps, approximately 1 μm in diameter, permitted emigration of erythrocytes whose outline appearing a mushroom-like protrusion into the pericapillary space, and the basal lamina still remained its structural integrity covering the deformed erythrocytes (Fig. 3). At this stage visible structural damage of the endothelial cells was not encountered.

In the second type of extravasation, rather predominant, erythrocytes migrated through the intercellular tight junction between adjacent endothelial cells (Figs. 4 and 5). The most characteristic feature of the migrating red blood cells was an extremely irregular configuration of the cell when pushed it through the junction. Constriction of the erythrocyte by the endothelial junction was so tight that the migrating cell body at the level of the junction was extremely thin and thread-like contour of less than 100 μm in diameter (Figs. 4 and 5). Extravasated erythrocytes adhered directly to the parenchymal glandular cells without intervening basal laminae.

Subsequently some blood capillaries contained almost no cellular components of the blood, whereas their endothelial cells were intact in fine structure (Fig. 6). Close examination revealed that the narrow pericapillary space was provided with a double structure of the continuous basal laminae (Fig. 6, inset). The extravasated erythrocytes had a tendency to gather in groups forming a number of massive conglomerates among the parenchymal glandular cells. These erythrocytes moved, without exception, towards the hypophyseal residual lumen after 20 min of the injection (Fig. 7). The conglomerates creeped into the intercellular space of the marginal cells whose apical zone was provided with distinct tight junctions (Fig. 7). Eventually the erythrocytes of conglomerates began to migrate into the Rathke's residual lumen through the zonula occludens of the marginal cells (Fig. 8). As was the case of erythro-

FIG. 1. Extravasation of fragmented erythrocytes into the intercellular space of the parenchymal cells between two blood capillaries filled with red blood cells 10 min after ip injection of 9% NaCl. No visible structural lesion of endothelial cells is evident. Scale bar: 1 μm.

FIG. 2. A small protrusion (arrow) of erythrocytes (Er) penetrating into the endothelial gaps (or stomata) 10 min after 9% NaCl injection. The opposing two basal laminae (arrow heads) in the pericapillary space are intact. E, endothelial cells. Scale bar: 1 μm.

FIG. 3. Dilated endothelial gaps through which migration of red blood cells showing mushroom-like-extrusion that is invested with intact basal lamina (arrow head) after 10 min of 9% NaCl injection. E, endothelial cells. Scale bar: 1 μm.

FIG. 4. Red blood cells emerging from capillaries through the endothelial junctions 10 min after 9% NaCl injection. Irregular shape of erythrocyte showing thread-like configuration at the level of the junction. No structural destruction of endothelial cells (E). Scale bar: 1 μm.

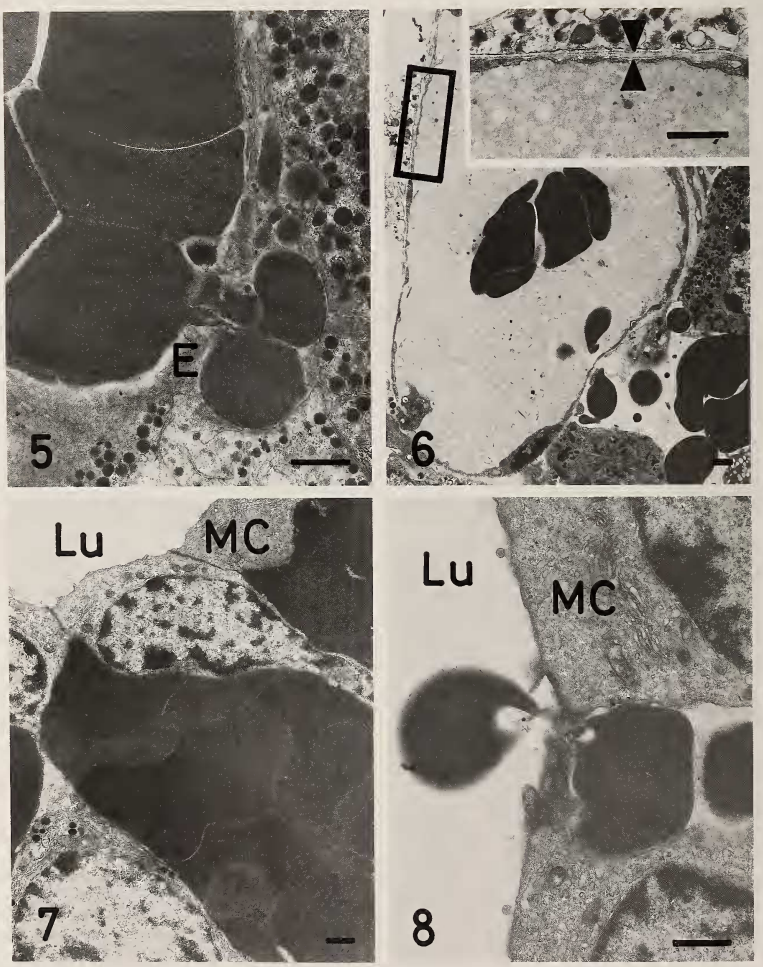

FIG. 5. Another erythrocyte emigration through endothelial junctions 10 min after 9% NaCl injection. Note the extreme constriction of the cell body when passing through the junction. E, endothelial cells. Scale bar: 1 μm.

FIG. 6. Blood capillary without structural lesion and its lumen containing little cellular components 20 min after 9% NaCl injection. Scale bar: 1 μm. *Inset*, Higher magnification of a portion of pericapillary space supported by two intact basal laminae (arrow heads). Scale bar: 1 μm.

cytes diapedesis (Figs. 4 and 5), the traversing erythroctes squeezed through the tight junction of marginal cells and showed thread-like configuration in the middle of the cell body (Fig. 8). Additionally the maximum extent of hemorrhage into the anterior pituitary was observed 12 hr after the injection of hypertonic solution and lasted for approximately 3 days [1].

DISCUSSION

Hemorrhage from pituitary adenoma is known as "pituitary apoplexy", a clinical condition that has been first described by Bleibtreu in 1905 [6]. Many factors and possible mechanism by which hemorrhage could occur in pituitary adenoma have been proposed in cases including bromocriptine therapy [7, 8], radiation therapy [9], anticoagulant therapy [10], atherosclerotic embolization [11, 12], thrombosis of the hypophyseal portal vein [13], induction of ovulation [14], head trauma [15], spontaneous necrosis of pituitary tumors [16], carotid angiography [17], arteriography [18] and pre-operative test for pituitary function [19]. The pathogenesis of pituitary apoplexy may not be uniform, but individual cases are probably caused by different mechanism [8]. Whatever the mechanisms are involved, pituitary apoplexy is a life-threatening disorder that requires prompt medical treatment to avoid catastrophe [12]. To obtain an awareness of predisposing factors it seems necessary to establish an experimental system which mimics human pituitary apoplexy. In this regard, our previous studies have demonstrated acute and intense hemorrhage occurring exclusively in the anterior pituitary after ip injection of hypertonic solutions in the conscious mouse [1]. The present electron microscopic study has accumulated an additional evidence that anterior pituitary hemorrhage occurs without obvious destruction of sinusoidal blood capillaries, that is a mechanism known as diapedesis. Up to date no reports have been available dealing with the earliest ultrastructural changes in the erythrocyte diapedesis of either pituitaries or other endocrine glands. Electrolytic destruction of the pituitary stalk resulted in the disrupted capillary walls followed by the leakage of red blood cells and platelets in the pituitary of the rat [2]. Acrylonitrite administration [3] and chronic ACTH stimulation [4] caused hemorrhage into the adrenal cortical zone in rats but no initial changes of erythrocyte extravasation were illustrated.

Under the present experimental conditions the observed erythrocyte diapedesis in the anterior pituitary can be accounted for by postulating the two passages of traversing endothelial cells: through the large endothelial gaps up to 1 μm in diameter, and through the occluding intercellular junctions between adjacent endothelial cells. The fenestrated capillaries of the endocrine gland type, unlike sinusoids of the hepatic type, are not provided with the large endothelial gaps. Thus these endothelial gaps observed were formed under the present experimental conditions. It is suggested, therefore, that the presence of large endothelial gaps may represent a modification of endothelial junctions as reported after leukotriene B_4 treatment in leukocyte diapedesis in the rabbit [20]. Alternatively, another possibility that endothelial fenestrae, a regular feature of endocrine glands, gather and fuse together to form large endothelial gaps or stomata under the given conditions should be considered. In fact the expansion of stomata is now believed to be a cause of diapedesis in cases including hemorrhagic infarct in the lung, scurvy, purpura, leukemia, vicarious menstruation and toxipathic jaundice.

The second passage of erythrocytes for diapedesis occurred, predominantly, through the endothelial tight junction between adjacent cells (Figs. 4 and 5). In general, the zonula occludens is impermeable for free migration of substances along the intercallular space [21]. However, the junctional complex differs in precise arrangement from one organ to another. The proximal convo-

FIG. 7. Large conglomerates of erythrocytes locating beneath the marginal cells (MC) 20 min after 9% NaCl injection. Lu, hypophyseal lumen. Scale bar: 1 μm.

FIG. 8. Extreme constriction of red blood cells migrating into the hypophyseal lumen through the tight junction of marginal cells (MC). Scale bar: 1 μm.

luted tubule of the mouse kidney has a "very leaky epithelium" provided with the zonula occludens consisting in most places of only one junctional strand [22]. Further, the presence of "maculae occludentes" rather than "zonulae occludentes" has been demonstrated in the mouse cardiac muscle [23]. Although general features of the endothelial junctions of endocrine glands are not known, it is considered that erythrocyte diapedesis through endothelial junctions in the anterior pituitary after hypertonic solution administration may be ascribed to structural variations of the junctional complex of either "leaky" structure of the zonulae occludentes or the "maculae occludentes".

One of the interesting findings in this pituitary hemorrhage is the erythrocyte migration into the hypophyseal lumen through tight junctions of the marginal cells which are thought to be provided with a epithelial type of zonulae occludentes. Thus, the zonulae occludentes of the marginal cells may be made up of one or two junctional strands like those of the proximal tubules of the mouse kidney [22]. The similar configuration of red blood cells during the migration through the tight junctions of endothelial cells (Figs. 4 and 5) and of margnal cells (Fig. 8) favors the assumption that both of the tight junctions may belong to the type of zonulae occludentes of leaky in structure. The structural relationship between the endothelial tight junction and the endothelial gaps (or stomata) in diapedesis remains unclear. Further work is required to establish the fine structure and function of endothelial cell junctios of the anterior pituitary.

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